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Photochemical Oxidant Injury and Bark Beetle (Coleoptera: Scolytidae) Infestation of Ponderosa Pine

I. Incidence of Bark Beetle Infestation in Injured Trees

R. W. Stark, P. R. Miller, F. W. Cobb, Jr., D. L. Wood, and J. R. Parmeter, Jr.

II. Effect of Injury upon Physical Properties of Oleoresin, Moisture Content, and Phloem Thickness

F. W. Cobb, Jr., D. L. Wood, R. W. Stark, and P. R. Miller

III. Effect of Injury upon Oleoresin Composition, Phloem Carbohydrates, and Phloem pH

P. R. Miller, F. W. Cobb, Jr., and E. Zavarin

IV. Theory on the Relationships between Oxidant Injury and Bark Beetle Infestation

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Certain aspects of insect-disease relationships, especially those concerning transmission of pathogens, have been studied extensively and their significance has been well established. However, the role of diseases as factors predisposing coniferous trees to bark beetle infestation has received only minor attention. There has been little effort to determine the extent of the association between disease and bark beetle infestation, the significance of predisposing diseases in the ecology of the beetles, or the effects of disease upon the host that may increase susceptibility to beetle attack.

The series of papers in this issue presents the results of studies to determine (a) the degree of association between photochemical atmospheric pollution injury to ponderosa pine and infestation by bark beetles (paper I), and (b) the changes in the physiology of diseased trees which might influence host susceptibility to bark beetles (papers II and III). The results show that oxidant injury does, in fact, predispose ponderosa pine to beetle infestation, and that the injury leads to physiological changes in the host which may be related to increased bark beetle susceptibility. The significance of these results in relation to the present knowledge on bark beetle ecology and host susceptibility is discussed in paper IV.

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II. Effect of Injury upon Physical Properties of Oleoresin, Moisture Content, and Phloem Thickness[1]

INTRODUCTION

A HIGH DEGREE of association between disease caused by atmospheric pollution and incidence of bark beetle (Scolytidae) infestation in ponderosa pine, *Pinus ponderosa* Laws., has been demonstrated (paper I). Results also indicated that bark beetles can select severely diseased trees. Thus, an understanding of the influence of disease on host physiology may elucidate mechanisms of host resistance to bark beetles.

Studies were made during the summer, 1966, to determine the effects of atmospheric pollution injury (i.e., chlorotic decline) of ponderosa pine on various factors related to tree physiology. This paper reports the results of such studies on the following factors: (a) oleoresin exudation pressure; (b) yield and rate of flow; (c) crystallization rate; (d) sapwood moisture content; (e) phloem moisture content; and (f) phloem thickness.

The field aspects of the studies were carried out in the vicinity of Crestline in the San Bernardino Mountains. The area is about 1,550 meters above sea level at the northeastern edge of the Los Angeles basin. Photochemical air pollutants diffusing over the mountains from the basin have resulted in chronic, severe pollution injury to ponderosa pine.

METHODS

A second-growth, all-age stand of ponderosa pine with most trees 65–85 years old was chosen for the study. Because susceptibility to atmospheric pollutants varies in ponderosa pine, we were able to select 50 trees in each of three disease classes—healthy, intermediate-diseased, and advanced-diseased. Trees with disease ratings of 0–1, 2–4 and 5 or greater,[2] were classified as healthy, intermediate- and advanced-diseased, respectively. The rating of intermediate-disease was difficult, and overlap between this class and the two extremes occasionally occurred. Diameter, height, length of crown, and crown class were recorded for each tree. No suppressed trees were selected.

Measurement of oleoresin exudation pressure (OEP)

Standard hydrostatic pressure gauges (200 psi maximum) with specially constructed fittings (Bushing and Wood, 1964) were used to measure oleoresin exudation pressure of all trees. Holes approximately 13 mm. in diam-

[1] Submitted for publication June 15, 1967.
[2] See page 122 for scoring system.

eter and 3 cm deep were drilled into the boles of the trees 1 to 1.5 meters above ground. The gauges were immediately screwed into the holes to a depth of approximately 2.5 cm. The first pressure readings were taken the day following installation of the gauges. Previous studies (Vité, 1961) have shown that diurnal fluctuations in OEP occur. Thus, three readings per day were taken, the first before sunrise, approximately at 5 a.m., the second at noon, and the third at 4 p.m. The early morning reading was made when OEP was at its highest, and the two later readings provided an estimate of the OEP at its lowest point. Low OEP measurements were taken only from trees with a morning OEP of 80 psi or greater. A fourth reading was taken before sunrise on the second morning for comparison with the pressure on the first morning. If the reading did not rise at least 10 psi above the low of the previous day, the gauge was taken out and another was placed into the tree. Any gauge which did not read at least 80 psi at the first reading was removed and another was inserted into a freshly drilled hole. As many as five or six gauges were installed in the trees during a one-week period to obtain an accurate, replicated reading.

Measurement of oleoresin yield, flow rate and crystallization

Glass tubes were used to measure both resin yield and flow rate in each tree.

They consisted of two parts: (a) 9 mm outside and 1 mm inside diameter capillary tubing 15 cm long bent to a 90°angle at the center; and (b) a tube 20 cm long with an inside diameter of 9 mm. The latter tube was sealed at one end, and a small hole was made in the side wall 3 cm from the open end to allow escape of gases as resin collected in the tube. Two elbow tubes were positioned on opposite sides of each tree 1 to 1.5 m above ground. They were inserted into holes 1.5 cm deep and 8 mm in diameter to a depth of about 1 cm. One of the larger diameter tubes was then inserted over the open end of the elbow and taped into place (figure 1).

After installation, linear measurements were taken at 15 to 30 minute intervals for the first four to six hours to establish rate of flow. Less frequent measurements were made for 24 to 72 hours or until the tubes became filled. Flow rate was expressed as ml of oleoresin per hour, yield as ml of oleoresin collected during the first 24 hours. Measurements for the two tubes of each type were averaged unless the measurement for one of the tubes was obviously

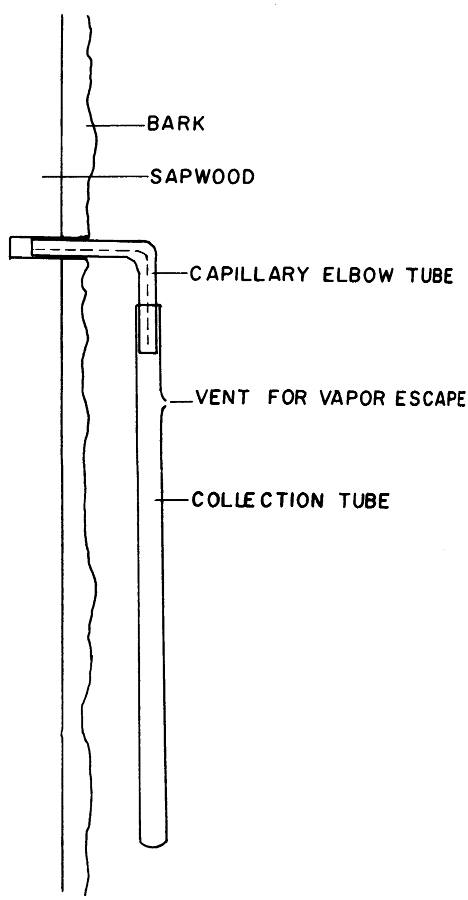

Fig. 1. Collection and capillary tubes used to determine oleoresin yield, flow rate, and crystallization rate.

inaccurate because of blockage. At the time of each measurement, the resin was carefully examined for the presence of crystals. The time of the first observation of crystal formation and an estimate of percentage of volume represented by crystals was recorded at that time and at all subsequent examinations.

Determination of sapwood and phloem moisture contents

Samples of sapwood and phloem were taken between 4 and 7 p.m. from four sides of each tree, 1 to 1.5 m above ground. Sapwood samples 4 cm in diameter and 5 to 8 mm thick were cut with an arch punch, removed with a chisel, and wrapped immediately in aluminum foil to prevent water loss. The foil-wrapped samples were weighed two to four hours after collection; then the foil was removed, dried and weighed. The samples were dried at approximately 100°C for 12 to 24 hours and weighed. The moisture content, expressed as percentage of dry weight, was then calculated by dividing the weight of water in the fresh sample by the dry weight of the sample.

The phloem samples, approximately 4 cm in diameter, were removed from the trees with a knife following removal of the bark and wrapped in aluminum foil. Fresh weight and moisture content were determined as for sapwood but moisture content was also determined on a percentage of saturation basis (Bier, 1959).

Measurement of phloem thickness

Samples of phloem were taken from all trees with an increment borer and the phloem thickness measured to the nearest half-millimeter. In February, 1967, phloem measurements from an additional 60 trees in a nearby, comparable stand were made in the same manner. The latter sample included 20 trees in each disease class.

RESULTS AND DISCUSSION

Relation of tree characteristics to disease severity

Diameter at breast height ranged from 24 to 93 cm, and total height ranged from 11 to 36 meters. Disease severity appeared to be related to the differences in tree size (table 1). The ratio of total crown length (living plus dead) to tree height was approximately the same for trees in all three disease classes. However, the live crown ratios (length of living crown/total tree height) became progressively less as disease severity increased, thus reflecting the excessive mortality of branches in the lower crowns of diseased trees.

Oleoresin exudation pressure

The oleoresin exudation pressure was progressively reduced in ponderosa pine as severity of injury by atmos-

TABLE 1
RELATION OF TREE CHARACTERISTICS TO SEVERITY OF DISEASE CAUSED BY PHOTOCHEMICAL AIR POLLUTION

Disease category	Average diameter	Average height	Live crown ratio	Crown class: Dominant	Co-dominant	Intermediate
	cm	*m*	*Per cent*	*Number of trees*		
Healthy	50	25	56	19	28	3
Intermediate	44	23	52	6	36	8
Advanced	39	20	35	3	30	17

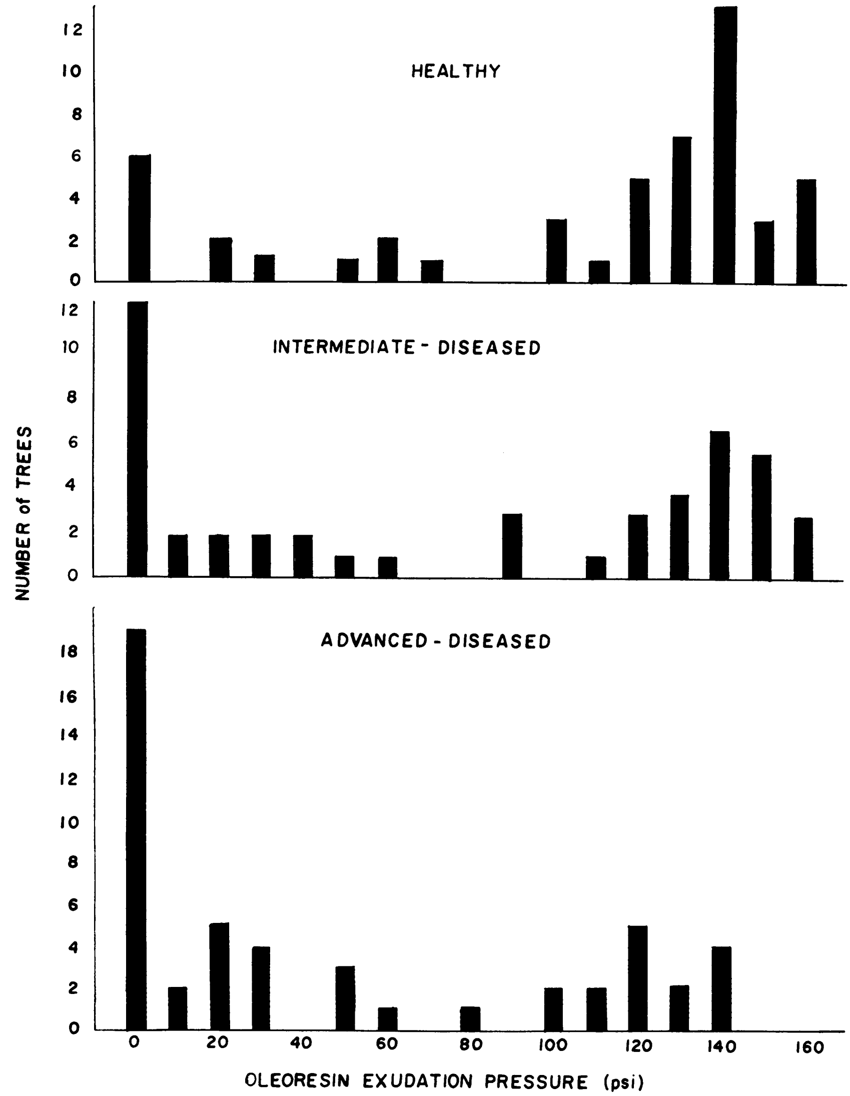

Fig 2. Distribution of ponderosa pines by OEP in relation to severity of disease caused by photochemical atmospheric pollution.

pheric pollution increased (figure 2). Some zero-pressure and low-pressure trees can be expected in a natural population of healthy ponderosa pines (Vité and Wood, 1961). Six of the healthy trees in our study had zero OEP, and an additional seven had low pressures (5–75 psi). However, the number of zero-pressure trees increased to 13 in the intermediate-diseased group and to 19 in the advanced-diseased group. The number of low-pressure trees also increased as disease severity increased, and there was a corresponding decrease in the number of high-pressure trees (80 psi and above). The average high OEP of all healthy, intermediate-diseased and advanced-diseased trees was 105, 78 and 46 psi, respectively. A χ^2 test on the differences between the distribution of trees in the healthy and intermediate-diseased

TABLE 2

RELATION OF LOW OEP AND OEP DEPRESSION TO INJURY OF PONDEROSA PINES BY PHOTOCHEMICAL AIR POLLUTION

Disease class	Number of trees* tested	Percentage with valid low	Average low OEP†	Average depression†
			psi	*psi*
Healthy	37	76	94	47
Intermediate	27	52	93	46
Advanced	16	38	80	38

* The low OEP was determined only for trees with an early morning high OEP of 80 psi or greater.
† The average low OEP and the average depression of OEP are based on the number of trees for which a valid low OEP was obtained.

TABLE 3

RELATION BETWEEN MAGNITUDE OF THE HIGH OEP (80 PSI AND GREATER) AND SUCCESS IN OBTAINING A RELIABLE LOW MEASUREMENT FOR PONDEROSA PINES INJURED BY PHOTOCHEMICAL AIR POLLUTION

Disease class	High OEP (psi)							
	80–100		101–120		121–130		131–160	
	Valid low	No low	Valid low	No low	Valid low	No low	Valid low	No low
	Number of trees							
Healthy	0	3	1	2	6	3	21	1
Intermediate	1	2	1	3	1	2	11	6
Advanced	0	3	2	5	0	1	4	1
Total	1	8	4	10	7	6	36	8

groups and between the intermediate- and advanced-diseased groups was significant at the 99 per cent confidence level ($\chi^2 = 12.11$ and 9.75, respectively).

The effect of air pollution injury on the average low OEP and magnitude of the diurnal depression was determined for all trees with a high OEP of 80 or more (table 2). While the average diurnal low OEP of trees in the advanced-diseased group was 13–14 psi less than that in the healthy or intermediate-diseased groups, the magnitude of depression was also less. This can be related directly to the lower "high" OEP of advanced-diseased trees which was 118 compared to 141 and 139 psi for trees in the healthy and intermediate-diseased groups, respectively. Because trees with an OEP of less than 80 psi were not included and trees in the 80–120 psi range yielded relatively few valid lows (table 3), the averages in table 2, which are based only on valid lows, are higher than those for the original population.

Differences in the numbers of trees yielding valid low readings may have significance. Greater success was achieved in determining the low readings of trees with an OEP of 131 psi or more (table 3). Also, when the intermediate-and advanced-diseased trees were grouped (OEP 131 and >) and compared to the healthy trees, success in determining low pressures was 68 and 95 per cent for the diseased and healthy groups, respectively. This difference could be caused by increased propensity of oleoresin from diseased trees to crystallize.

TABLE 4

OLEORESIN YIELD DURING THE FIRST 24-HOUR PERIOD IN RELATION TO INJURY OF PONDEROSA PINES BY PHOTOCHEMICAL AIR POLLUTION

Disease class	Total number of trees	Resin yield (ml)					Average resin yield
		0	0.1-1.9	2.0-3.9	4.0-5.8	5.9 (full)	
		Number of trees					*ml*
Healthy	21	0	6	2	2	11	4.1
Intermediate	19	1	2	3	4	9	4.2
Advanced	19	2	8	6	3	0	2.1

TABLE 5

RELATION OF OLEORESIN FLOW RATE OF PONDEROSA PINES TO INJURY BY PHOTOCHEMICAL AIR POLLUTION

Disease class	Total number of trees	Resin flow rate ml/hr					Average rate of flow
		0-.2	.3-.5	.6-1.0	1.1-1.5	1.6	
		Number of trees					*ml/hr*
Healthy	20	7	6	3	3	1	0.6
Intermediate	19	7	7	1	3	1	0.6
Advanced	19	12	7	0	0	0	0.2

Oleoresin yield, rate of flow, and crystallization rate

These characteristics were determined for 21 healthy trees and 19 trees in each of the two classes showing disease symptoms.

The yield of more than 50 per cent of the trees in the advanced-diseased group was less than 2.0 ml compared to 16 per cent and 29 per cent of the intermediate-diseased and healthy trees, respectively (table 4). Conversely, more than 60 per cent of the healthy and intermediate-diseased trees yielded more than 4.0 ml of resin compared to only 15.8 per cent of the advanced-diseased trees.

The average yields for trees in the healthy, intermediate-diseased and advanced-diseased classes were 4.1, 4.2, and 2.1 ml, respectively. The averages for the first two groups were conservative because the value of 5.9 ml was assigned to all filled tubes; tubes on 11 of the healthy and nine of the intermediate-diseased trees were filled before the end of the 24-hour period. There was no significant difference between the yield of healthy and intermediate-diseased trees. However, the difference between yields of these two groups and that of advanced-diseased trees was highly significant (0.01 level; $F = 7.64$, $df = 58$).

The rate of resin flow had a trend similar to resin yield (table 5). No difference existed between the flow rate of healthy and intermediate-diseased trees, but the flow rate of advanced-diseased trees was significantly reduced (0.05 level; $F = 3.70$, $df = 57$).

Unlike oleoresin exudation pressure which apparently was reduced gradually as severity of disease increased, flow rate appeared to remain relatively constant through the intermediate stages of disease development. No difference in oleoresin yield appeared to exist between healthy and intermediate-

TABLE 6

OLEORESIN CRYSTALLIZATION OF PONDEROSA PINES DURING THE FIRST 24 HOURS IN RELATION TO INJURY BY PHOTOCHEMICAL AIR POLLUTION

Disease class	Total number of trees	Crystallization (per cent)					Average crystallization
		0–5	6–10	11–20	21–40	41 >	
		Number of trees					*Per cent*
Healthy	21	13	4	2	2	0	7.3
Intermediate	18	5	6	6	1	0	9.7
Advanced	17	7	2	3	4	1	13.9

diseased trees but, because of the limited capacity of the collection tubes, this cannot be stated with certainty.

Crystallization of oleoresin increased with increase in disease severity (table 6). Greater than 10 per cent crystallization of oleoresin occurred in samples from 47 per cent of the advanced-diseased trees, 39 per cent of the intermediate-diseased trees and only 19 per cent of the healthy trees. The difference between the amount of crystallization in samples from healthy and advanced-diseased trees was highly significant (0.01 level; $F = 13.93$, $df = 37$). However, differences between healthy and intermediate-diseased and between intermediate-diseased and advanced-diseased trees were not significant. The wide range of variability and relatively small sample size may account for the absence of significant differences between these classes.

Sapwood and phloem moisture

The moisture content of both sapwood and phloem tissues showed a marked decrease in both classes of diseased trees (table 7). The differences in sapwood moisture content between healthy and intermediate-diseased trees ($F = 16.0$, $df = 29$) and between healthy and the advanced-diseased trees ($F = 10.4$, $df = 29$) were significant at the 0.01 level. However, the difference in sapwood moisture content between intermediate-and advanced-diseased trees was not significant. Thus, much of the moisture decrease in sapwood apparently occurred early during disease development. Reduction in water loss by transpiration associated with reduced foliage of severely diseased trees may have been a factor.

The differences in phloem moisture content, based on dry weight, between healthy and intermediate-diseased ($F = 6.56$, $df = 31$) and between healthy and advanced-diseased trees ($F = 16.5$, $df = 32$) were also highly significant; again, the difference between intermediate and advanced-diseased trees was not significant. However, when phloem moisture content was determined on the percentage saturation basis, the difference between the intermediate-diseased and advanced-diseased groups

TABLE 7

RELATION OF PONDERSOA PINE SAPWOOD AND PHLOEM MOISTURE CONTENT TO INJURY BY PHOTOCHEMICAL AIR POLLUTION

Disease class	Sapwood moisture*	Phloem moisture†	
	Per cent dry weight	*Per cent dry weight*	*Per cent saturation*
Healthy	138.2	287.2	64.8
Intermediate	109.9	263.6	61.7
Advanced	116 7	252.7	54 4

* Averages of four samples from each of 15 trees per disease class.

† Averages of four samples per tree from 16 trees in the healthy and intermediate-diseased classes and from 17 trees in the advanced-diseased class.

proved also to be highly significant (0.01 level; F = 4.9, df = 32). Possibly, phloem from trees with severe disease symptoms is more porous and can absorb more water per unit weight of tissue.

Phloem thickness

Phloem thickness decreased progressively with increase in disease severity. The average thickness of the phloem of healthy trees, measured in 1966, was 2.4 mm (range 1 to 6 mm); that of intermediate-diseased trees was 1.9 mm (range 1 to 4 mm); and that of advanced-diseased trees was 1.5 mm (range 1 to 5 mm). These differences were highly significant between healthy and intermediate (F = 8.0, df = 99) and between intermediate- and advanced-diseased trees (F = 36.7, df = 99). Results from the second plot measured in February, 1967, substantiate these differences. The average phloem thickness of healthy trees again was 2.4 mm (1.5 to 4.0), that of intermediate-diseased trees was 1.4 mm (1.0 to 2.5), and that of advanced-diseased trees was 1.1 mm (0.5 to 2.0).

SUMMARY

The results of these studies show that disease caused by photochemical atmospheric pollution affects certain physiological properties of ponderosa pine that may be related to increased susceptibility to bark beetles. Oleoresin exudation pressure, yield, and rate of flow were substantially reduced in severely affected trees, but crystallization of resin increased as severity of disease became greater. Both sapwood and phloem moisture contents were less in diseased trees. Phloem thickness in advanced-diseased trees was less than 60 per cent of that in healthy trees. The significance of these differences associated with oxidant injury is discussed in relation to host susceptibility to bark beetles in paper IV of this Hilgardia.

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